Competent Cell Preparation

This protocol is used for the preparation of chemically competent Escherichia coli. It is important to make all the measurements correctly.

1. Start by making a fresh streaking of the bacteria in a LB with agar plate. Incubate overnight (ON) at 37°C.
2. Dissolve a single colony from the plate in 20ul of sterile water, and add it to 5ml of LB to make a liquid culture. Shake at 220 rpm until the Optical Density at 600nm is close to 0,5 (6h approx.).
3. Take 5ml of the culture and add them to a erlenmeyer flask with 50ml of LB. Shake at 220rpm until the Optical Density at 600nm is close to 0,5 (4h approx.).
4. Centrifuge 30 minutes at 3000 rcf at 4°C.
5. Discard the liquid phase and add 25ml of CaCl2 0,1M. Resuspend the bacterial pellet by pipetting carefully.
6. Incubate at 4°C for more than 12h and less than 16h.

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7. Centrifuge 30 minutes at 3000 rcf at 4°C.
8. Discard the liquid and add 2ml of CaCl2 with 15% glycerol, resuspend the cells.
9. Measure aliquots of 50ul and add them to eppendorf tubes. Store at -80°C.